## REMARKS

Claims 41-69 are pending and have been examined in the present Office action. Claims 44-69 were rejected under 35 U.S.C. § 112, first paragraph, for failure to comply with the written description requirement. Claims 41-69 were rejected under 35 U.S.C. § 112, for an asserted lack of enablement. Claims 45-47 and 50-67 were rejected under 35 U.S.C. § 112, second paragraph, for indefiniteness. Claims 43, 45, 46, 49, 56-60, and 62-65 were also rejected under 35 U.S.C. § 102(b). Each of these rejections is addressed below.

### The Invention

The claimed invention features methods for diagnosing pre-eclampsia and eclampsia or a propensity to develop pre-eclampsia or eclampsia, that include detecting the levels of sFlt-1, free PlGF, or free VEGF in a pregnant human subject. Pre-eclampsia is a syndrome that affects 5 to 10% of pregnancies and results in substantial maternal and fetal morbidity and mortality. Applicants have discovered that levels of the soluble form of the Flt-1 receptor (sFlt-1) for VEGF and PlGF are increased in subjects having or at risk for developing pre-eclampsia or eclampsia. According to Applicants' model, during pre-eclampsia, levels of sFlt-1 are increased and can complex to PlGF and VEGF, thereby reducing the levels of free PlGF and free VEGF in a subject having pre-eclampsia or eclampsia. As a result, diagnostic methods that include the detection of sFlt-1, free PlGF, or free VEGF polypeptides can be used to diagnose pre-eclampsia or eclampsia.

Amendments to the Claims

As an initial matter, Applicants thank Examiner Dang and Supervisor Brenda Brumback for the productive telephonic interview conducted on November 6, 2006. Applicants have amended the claims as discussed during the interview. In addition, as requested, we review below matters discussed in the interview.

Claims 1-34, 51-53, 57, and 69 are cancelled. Claims 35-40 are withdrawn. Claims 41-45 and 50 have been amended to recite the limitation that the subject is a human subject. Support for this amendment is found throughout the specification and the claims, for example, in claim 57 and at page 7, lines 10-13. Claims 42, 43, 45, 48, and 50 have been amended to recite the limitation that "said free PlGF is a PlGF polypeptide that has the ability to bind to sFlt-1." Claims 44, 45, 48, and 50 have been further amended to recite the limitation that "said free VEGF is a VEGF polypeptide that has the ability to bind to sFlt-1." Support for these amendments is found throughout the specification, for example, at page 28, lines 1-6. Applicants note that these limitations are included to clarify the functional attributes of the claimed VEGF or PlGF polypeptide. These amendments are not intended to limit the claim to the bound species of VEGF or PlGF; rather the claims require detection of the free species of VEGF or PlGF. Claims 45 and 50 to recite the limitation that an increase of at least 10% in the level of sFlt-1 or a decrease of at least 10% in the level of free VEGF or free PlGF

relative to a reference diagnoses the subject as having or having a propensity to develop pre-eclampsia or eclampsia. Support for this amendment is found throughout the specification, for example, at page 11, lines 22-24, and page 51, lines 19-22.

Claims 46, 47, and 49 include the use of the metric and comparing the metric to a reference sample. Applicants have amended claims 46, 47, and 49 to include the limitations that a difference of at least 10% between the subject metric and the reference metric diagnoses the subject as having or having a propensity to develop pre-eclampsia or eclampsia. Support for these amendments is found throughout the specification, for example, in previous claims 46, 47, 51, 52, and 53 and at page 11, lines 22-24 and page 51, lines 19-22. Claim 68 has been amended to depend from claims 45, 49, or 50 and to include mild pre-eclampsia, severe pre-eclampsia, or pre-eclampsia-associated HELLP, IUGR, or SGA. Support for this amendment can be found, for example, in Figures 1A-1C and 7A-7B, and at page 16, lines 18-26; page 18, line 7 to page 19, line 3; page 23, line 24 to page 24, line 12; page 26, line 27 to page 27, line 4; page 28, line 23 to page 29, line 1; and page 46, line 22 to page 48, line 18.

New claims 70-81 have been added. Claims 70-72 depend from claims 41, 45, 49, and 50 and recite the limitation that the sFlt-1 is free, bound, or total sFlt-1. Support for this limitation is found throughout the specification, for example, at page 7, lines 13-15. New claims 73-74 depend from claims 45 or 50 and recite the limitation that the increase in the level of sFlt-1 is at least 50% or 90% and that the

decrease in the level of free VEGF or free PlGF is at least 50% or 90%. New claim 75-76 depend from claim 47, and recite the limitation that the increase in the PAAI is at least 50% or 90%. New claims 77-78 depend from claim 49 and recite the limitation that the increase in the sFlt-1/PlGF is at least 50% or 90%. Support for these claims is found throughout the specification, for example, at page 11, lines 22-24 and page 51, lines 19-22. New claim 79 depends from claims 42 or 43 and recites the limitation that the method further includes measuring sFlt-1 levels. New claim 80 depends from claim 45 and recites the limitation that the method includes measuring sFlt-1 and PlGF levels. Support for these claims are found, for example, at page 6, lines 14-16, and page 45, line 12 to page 48, line 11. New claim 81 depends from claim 66 and recites the specific types of cells. Support for this claim is found in previous claim 64. No new matter has been added.

Rejections under 35 U.S.C. § 112, first paragraph

Claims 41-69 are variously rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the written description and enablement requirements. Each of the rejections is addressed below.

*Written Description*

Claims 44-69 stand rejected under § 112, first paragraph, because, according to the Examiner, the claims contain subject matter that was not described in the specification in such a way as to reasonably convey to one skilled

in the relevant art that Applicants, at the time the application was filed, were in possession of the claimed invention. Applicants respectfully submit that, in view of the present amendment, this rejection can be withdrawn.

The written description requirement may be met by (MPEP § 2163) "a disclosure of sufficiently detailed, relevant identifying characteristics which provide evidence that Applicant was in possession of the claimed invention, i.e., complete or partial structure, other physical and/or chemical properties, functional characteristics when coupled with a known or disclosed correlation between function and structure, or some combination of such characteristics."

Claims 44, 45, 48, and 50, and the claims that depend therefrom, feature a method of diagnosing pre-eclampsia or eclampsia in a subject that includes detecting the level of free VEGF in a sample from the subject. Applicants have amended claims 44, 45, 48, and 50 to recite the limitation that the free VEGF is "a VEGF polypeptide that has the ability to bind to sFlt-1." Support for this limitation can be found, for example, at page 4, lines 22-25, and page 28, lines 1-6, where the specification states, "[w]hile the detailed description presented herein refers specifically to sFlt-1, VEGF, or PlGF, it will be clear to one skilled in the art that the detailed description can also apply to sFlt-1, VEGF, or PlGF family members, isoforms, and/or variants, and to growth factors *shown to bind sFlt-1*." (Emphasis added.)

As discussed during the interview, Applicants intend the limitation to capture VEGF fragments and isoforms as outlined in the specification for example

at page 4, lines 22-25. Applicants note that the limitation is intended to provide relevant identifying characteristics for VEGF. The VEGF detected is still the free form of VEGF that *has the ability* to bind to sFlt-1. Furthermore, as noted during the interview, one skilled in the art at the time the invention was filed would clearly understand that numerous VEGF polypeptides can bind to sFlt-1 and would know how to assay for VEGF polypeptides that can bind to sFlt-1 using techniques known in the art.

With regard to the Examiner's statement that there is some variability in the degree of potency in the VEGF family members with respect to the induction of proliferation of endothelial cells, Applicants submit that, as described during the interview, it is the ability of the VEGF to bind to sFlt-1 that is the critical characteristic of the VEGF as claimed in the methods of the invention. The present invention is based on Applicants' discovery that levels of sFlt-1, a receptor for VEGF and PlGF, are increased in subjects with pre-eclampsia or eclampsia. When the levels of sFlt-1 are increased, sFlt-1 can complex to PlGF and VEGF, thereby reducing the levels of free PlGF and free VEGF (see, for example page 3, lines 9-17). The ability of VEGF to bind to sFlt-1 is a critical characteristic of VEGF family members useful in the claimed methods and this activity is described throughout the specification, for example, at page 2, lines 18-24; page 3, lines 9-12; page 20, lines 13-16; page 27, lines 15-22; and page 46, lines 22-26.

In sum, Applicants submit that the amendment to claims 44, 45, 48, and 50, when combined with the description provided in the specification and the

extensive knowledge in the art regarding VEGF family members and their structure and function, particularly with respect to sFlt-1 binding function, is sufficient to convey to the skilled artisan that Applicants were in possession of the claimed methods at the time the application was filed. Applicants respectfully request that the rejection of claims 44-69 under 35 § U.S.C. 112, first paragraph, with regard to written description be withdrawn.

*Enablement*

The Examiner sets forth four different reasons for the rejection of claims 41-69 for lack of enablement. Applicants traverse this rejection in the context of the present amendment and submit that the application meets the enablement requirement with regard to amended claims 41-69.

*Amended claims 41-45 and 50 are limited to human subjects*

In the first basis for the rejection, the Examiner has rejected claims 41-69 for an asserted lack of enablement for non-human subjects including a cow, horse, sheep, pigs, dogs, or cats. As proposed during the interview, in order to bring the claims into alignment with the invention as elected in response to the Restriction Requirement mailed on February 22, 2006, Applicants have amended claims 41-45 and 50 to recite "human subject." All of the remaining claims depend from each of these claims. This basis for the rejection can be withdrawn.

*sFlt-1, VEGF, and PlGF are present in amniotic fluid*

In the second basis for the rejection, the Examiner rejects claims 41 and 45-59 for an asserted lack of enablement for measuring PlGF, sFlt-1, or VEGF levels in amniotic fluid or cerebrospinal fluid. While not agreeing with the Examiner's position, in order to expedite prosecution, Applicants have cancelled claims 40-43 and amended claim 65 to remove the reference to cerebrospinal fluid. With regard to the use of amniotic fluid as the sample in the claimed methods, Applicants submit that, as discussed during the interview, practice of the claimed diagnostic methods that fall within the scope of claims 41, 48, and 50, and the claims that depend therefrom, using amniotic fluid as the bodily fluid sample was enabled by the specification and the state of the art at the time the application was filed.

As stated during the interview and in the attached Declaration of Dr. Karumanchi, amniotic fluid is obtained through amniocentesis, a procedure that is standard in the art and is routinely performed by the clinician. The techniques for the detection of sFlt-1, free PlGF, and free VEGF described in the specification for bodily fluids, such as serum or urine, can be easily adapted for the detection of these polypeptides in amniotic fluid. The adaptation of the techniques described in the specification for the detection of the polypeptides in amniotic fluid, which is readily obtained by the skilled clinician, does not constitute undue experimentation.

With regard to the Examiner's question as to whether these growth factors can cross the barriers isolating the fluids, Applicants respectfully submit that it

was known in the art that sFlt-1, PlGF, and VEGF could be detected in amniotic fluid. As attested to by Dr. Karumanchi during the interview and in the attached Declaration, Banks et al., *Molecular Human Reproduction* 4:377-386 (1998) and Hornig et al., *J. Immunol. Methods* 226:169-177 (1999), attached to the Declaration as Exhibits A and B, respectively, describe the detection of sFlt-1 in amniotic fluid sample from women with normal pregnancies undergoing amniocentesis. Vuorela-Vepsalainen et al., (*Hum. Reprod.* 14:1346-1351 (1999)), attached herein as Exhibit C, describes the detection of VEGF and PlGF in amniotic fluid using an ELISA. These three articles demonstrate that sFlt-1, PlGF, and VEGF have been detected in amniotic fluid and provide evidence that these polypeptides can cross the barriers isolating the fluid. In view of the amendments and arguments presented herein, Applicants respectfully request that this basis for the rejection be withdrawn.

*HELLP, IUGR, and SGA are subsets of pre-eclampsia*

In the third basis for the enablement rejection, the Examiner rejects claims 68 and 69 on the basis that the specification, while enabling for the diagnosis of pre-eclampsia or eclampsia, does not reasonably provide enablement for the diagnosis of HELLP, IUGR, or SGA.

Applicants have amended claim 68 to depend from claims 45, 49, or 50 and to recite that the subject is further diagnosed as having or having a propensity to develop mild pre-eclampsia, severe pre-eclampsia, or pre-eclampsia-associated

HELLP, IUGR, or SGA. By way of this amendment, Applicants have clarified that claim 68 is directed to methods of further diagnosing the subject with any one of these subsets of pre-eclampsia. As attested to by Dr. Karumanchi during the interview, the diagnosis of mild pre-eclampsia, severe pre-eclampsia, or pre-eclampsia-associated HELLP, IUGR, or SGA is known in the art and is performed clinically using a standard set of symptoms. Each of the claimed subsets of pre-eclampsia and the accompanying symptoms is also described in the specification. (See, for example, page 16, lines 18-26; page 18, lines 17-25; page 28, line 23 to page 29, line 1; and page 41, lines 18-20.) In one example discussed during the interview, HELLP is diagnosed by hemolysis, elevated liver enzymes, and low platelet count. In view of the amendments and arguments presented herein, Applicants respectfully request that this basis for the rejection be withdrawn.

*sFlt-1, VEGF, and PlGF are present in endothelial cells, leukocytes, monocytes, and cells derived from the placenta*

In the fourth basis for the rejection, the Examiner rejects claims 41, 44, 45, 40, 64, and 66, because the specification, while enabling for urine and serum, does not reasonably provide enablement for measuring PlGF, sFlt-1 or VEGF levels in endothelial cells, leukocytes, monocytes, and cells derived from the placenta. In view of the amendments to the claims and the data provided in the attached Declaration of Dr. Karumanchi, Applicants submit that this basis for the rejection can be withdrawn.

The independent claims, as amended herein, can be broadly grouped into two categories. The first category (claims 41, 42, 43, 44, and 48) includes claims that are directed to methods of diagnosing pre-eclampsia or eclampsia by determining the absolute threshold level, which would be diagnostic of pre-eclampsia or eclampsia, for sFlt-1, free PlGF, or free VEGF, or metrics including these polypeptides. The second category (claims 45 and 50) includes claims that are directed to methods of diagnosing pre-eclampsia or eclampsia by determining the relative level of sFlt-1, free PlGF, or free VEGF, or metrics including these polypeptides as compared to a reference sample. Applicants have amended claims 66 and 67 to depend only from the claims that recite methods that include determining the relative level of sFlt-1, free PlGF, or free VEGF as compared to a reference sample (i.e., the second category described above). Therefore, the use of subject tissue or cells (e.g., endothelial cells, leukocytes, monocytes, and cells derived from the placenta) as the biological sample only pertains to methods where the levels of the polypeptide is compared to the level from a reference sample.

As attested to during the interview and stated in the attached Declaration of Dr. Karumanchi, a variety of cell types, including those listed in claim 66, have been shown in the art to express sFlt-1, VGEF, and PlGF. For example, Barleon et al., *Angiogenesis* 4:143-154 (2001), attached herein as Exhibit D, demonstrate the presence of sFlt-1 in human peripheral blood monocytes and in endothelial cells (ECs) from a variety of tissues including human dermal microvascular ECs,

human umbilical vein ECs, human kidney venous ECs, human kidney microvascular ECs, human renal microvascular ECs, and human umbilical artery ECs. In addition, as indicated by the nomenclature, VEGF, or vascular endothelial growth factor, is highly expressed in the endothelium, and PlGF, or placental growth factor, was initially isolated from placental tissue.

In addition, as attested to during the interview and in the attached Declaration of Dr. Karumanchi, the cells recited in claim 66 are readily obtained using techniques known to the skilled artisan. For example, placental cells are routinely obtained through chorionic villus sampling and monocytes are obtained through purification from peripheral blood mononuclear cells. Furthermore, the methods for detecting sFlt-1, free VEGF, and free PlGF described in the specification can be readily applied to the detection of these polypeptides in a cell or tissue sample and would not constitute undue experimentation.

Moreover, in the attached Declaration of Dr. Karumanchi, Applicants provide a working example of the detection and comparison of the levels of sFlt-1 in monocytes from a pre-eclamptic patient and a normotensive patient (see Exhibit E) using the methods described in the specification. This data provides a working example of the detection of one of the claimed polypeptides in one of the claimed cell types. A skilled artisan would understand that the same methods can be applied any cell type or tissue type in which the polypeptide is expressed.

In sum, Applicants have amended claims 66 and 67 to clarify that these methods depend only from the independent claims that include diagnosis of pre-

eclampsia or eclampsia based on relative levels of sFlt-1, free VEGF, or free PlGF. In view of these amendments, the description in the specification of the methods for detecting each of these polypeptides that can be applied to any cell or tissue, and the working example provided in the attached Declaration demonstrating the detection of a relative increase in sFlt-1 levels in monocytes from a pre-eclamptic subject as compared to a normotensive subject using the methods described, Applicants submit that this basis for the enablement rejection of claims 41, 44, 45, 50, 64, and 66 can be withdrawn.

Rejections under 35 U.S.C. § 112, second paragraph

Claims 45-47, 57-67, and 50-67 stand rejected under 35 U.S.C. § 112, second paragraph, as being indefinite. In particular, the Examiner states that claim 45 is indefinite because the claim does not recite a clear link between the method of diagnosing a subject with pre-eclampsia or eclampsia and what constitutes a diagnosis for pre-eclampsia or eclampsia. The Examiner further states that claims 50 and 51 are indefinite because the claims do not recite a clear determination as to what constitutes a significant increase in the level of sFlt-1 or a significant decrease in the level of free VEGF or free PlGF. The Examiner states that claims 52 and 54 are indefinite because the claims do not provide an indication regarding how an alteration or an increase in the PAAI value is indicative for the diagnosis of pre-eclampsia or eclampsia. Applicants respectfully submit that the rejection of claims 45-47, 57-67, and 50-67 is overcome by the present amendment.

Applicants have cancelled claims 51-53 and 57 and amended claims 45 and 50 to recite the limitation that an increase of at least 10% in the level of sFlt-1 or a decrease of at least 10% in the level of free VEGF or free PlGF relative to a reference diagnoses the subject as having or having a propensity to develop pre-eclampsia or eclampsia. Claims 46, 47, and 49 include the use of the metric and comparing the metric to a reference sample. For clarity, Applicants have amended claims 46, 47, and 49 to include the limitations that a difference of at least 10% between the subject metric and the reference metric diagnoses the subject as having or having a propensity to develop pre-eclampsia or eclampsia. In view of these amendments, Applicants respectfully submit that the rejection of claims 45-47, 57-67, and 50-67 for indefiniteness can be withdrawn.

Rejections under 35 U.S.C. § 102(b)

Claims 43, 45, 46, 49, 56-60, and 62-65 stand rejected under 35 U.S.C. § 102(b) as being anticipated by Charnock-Jones, WO 98/28006. According to the Examiner, Charnock-Jones teaches the detection of significantly lower concentrations of PlGF in pre-eclamptic women as compared to healthy controls. In addition, the Examiner states that Charnock-Jones teaches a method for a method for diagnosing pre-eclampsia that includes measuring the levels of at least two of sFlt-1, free VEGF, and PlGF polypeptides and that the levels can be compared to control subjects who are normotensive pregnant women. The Office also states that Charnock-Jones recites that the levels of PlGF and VEGF are

measured from plasma collected from women at different stages of gestation and measured using an immunological assays, such as an ELISA. Applicants respectfully disagree.

Charnock-Jones presents a model that is the exact opposite of Applicants' model with respect to sFlt-1 and VEGF levels in pregnancy. Charnock-Jones describes their invention as follows:

> Further by way of explanation, the present inventors have surprisingly found that soluble flt (sflt) is produced in large amounts by the placenta in women with normal pregnancies and that sflt can be detected in the serum of such women. Conversely, it is predicted that women with pre-eclampsia produce insufficient amounts of sflt, leading to the presence of excessive amounts of free, active VEGF. (Page 11, lines 25-29).

Charnock-Jones proposes a hypothetical model that is in direct opposition to the present invention because, according to Charnock-Jones, the levels of sFlt-1 are *decreased* in pre-eclampsia, which, based on the ability of sFlt-1 to bind to both VEGF and PlGF, would be predicted to result in *an increase* in the levels of free VEGF and free PlGF.

As described above, the inventors of the present application have discovered that levels of sFlt-1, a soluble form of the receptor for VEGF and PlGF, *are increased* in subjects with pre-eclampsia or eclampsia, and that sFlt-1 can complex to PlGF and VEGF, thereby reducing the levels of *free PlGF and*

*free VEGF* in a subject having pre-eclampsia or eclampsia or a predisposition to develop pre-eclampsia or eclampsia.

Turning to the claims, Charnock-Jones does not anticipate claims 45, 46, or 49 because each of these claims includes the detection of increased levels of sFlt-1 or decreased levels of free VEGF as diagnostic indicators of pre-eclampsia. As described above Charnock-Jones' model is in direct opposition to the present invention with respect to sFlt-1 and free VEGF because, according to Charnock-Jones, the levels of sFlt-1 are decreased in pre-eclampsia leading to an increase in the levels of VEGF.

With regard to the rejection of claim 43, Applicants respectfully submit that Charnock-Jones does not expressly describe the detection of *free* PlGF levels, as required by claim 43, and that the PlGF detected by Charnock-Jones would not necessarily be free PlGF and therefore does not inherently possess the same function and attributes as the free PlGF of the present claims.

As described throughout the present application PlGF, VEGF, and sFlt-1 are all present in the serum of a subject. Therefore, unless one was using a method of detection that was specific to the free form (e.g., an antibody specific to the free form), one would be unable to determine if the PlGF detected was free or bound to sFlt-1 or Flt-1. Therefore, detection of PlGF in a bodily fluid, does not inherently refer to the detection of *free PlGF*.

As attested to in the attached Declaration, the detection methods used to detect PlGF in the present invention specifically detected *free* PlGF. As described

in the attached Declaration, an assay using the ELISA kit described in the specification was performed using recombinant PlGF in the presence or absence of sFlt-1. The results are shown in Figure 2b of Maynard et al., (*J. Clin. Invest.* 111:649-658 (2003), attached to the Declaration as Exhibit F), which demonstrates that the level of PlGF detected by the ELISA significantly decreases in the presence of increasing levels of recombinant sFlt-1. The interference of sFlt-1 with PlGF measurement confirms that the PlGF detected is free PlGF and the decrease in the levels of PlGF detected in the presence of increasing levels of recombinant sFlt-1 is due to the fact that as sFlt-1 is added, more of the PlGF binds to sFlt-1 and is therefore not detected by the ELISA that is specific for only the free form of PlGF. These experimental results demonstrate that the detection methods used in the experiments described in the specification of the above-referenced application specifically detect the free form of PlGF.

Applicants submit that Charnock-Jones does not anticipate the methods of claim 43, and the claims that depend therefrom, because each of these claims requires that the detection of *free* PlGF, a limitation not taught, either expressly or inherently, by Charnock-Jones.

The remaining claims depend from independent claims 43, 45, 46, 49, and 50, and are therefore also not anticipated by Charnock-Jones because, by definition, these claims include all of the limitations of the independent claims.

In view of these distinctions between Charnock-Jones and the present claims, Applicants respectfully request that the rejection of claims 43, 45, 46, 49,

56-60 and 62-65 for anticipation by Charnock-Jones under 35 U.S.C. §102 (b) be withdrawn.

## CONCLUSION

Applicants submit that the claims are now in condition for allowance and such action is respectfully requested.

Enclosed is a Petition to extend the period for replying to the Office action for three months, to and including December 30, 2006, and a check in payment of the required extension fee. Also enclosed is a check for $700.00 in payment of excess claims fees for new claims.

Applicants note that the Form PTO 1449 that was submitted with an Information Disclosure Statement filed on October 25, 2006 has not been initialed and returned, and hereby request that it be initialed and returned with the next Office action.

If there are any additional charges or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

Date: December 7, 2006

Kristina Bieker-Brady, Ph.D.
Reg. No. 39,109

Clark & Elbing LLP
101 Federal Street
Boston, MA 02110
Telephone: 617-428-0200
Facsimile: 617-428-7045